Neuropsychopharmacology (2013) 38, 673–682

www.neuropsychopharmacology.org

Dopamine Response to Psychosocial Stress in Chronic Cannabis Users: A PET Study With [^{11}C]-(+)-PHNO

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A number of addictions have been linked with decreased striatal dopamine (DA) receptor availability and DA release. Stress has a key role in cannabis craving, as well as in modulation of dopaminergic signaling. The present study aimed to assess DA release in response to a laboratory stress task with [^{11}C]-(+)-PHNO positron emission tomography in cannabis users (CU). Thirteen healthy CU and 12 healthy volunteers (HV) were scanned during a sensorimotor control task (SMCT) and under a stress condition using the validated Montreal imaging stress task (MIST). The simplified reference tissue model (SRTM) was used to obtain binding potential (BP_{ND}) in striatal subdivisions: limbic striatum (LST), associative striatum (AST), and sensorimotor striatum (SMST). Stress-induced DA release (indexed as a percentage of reduction in [^{11}C]-(+)-PHNO BP_{ND}) between CU and HV was tested with analysis of variance. SMCT BP_{ND} was significantly higher in CU compared with HV in the AST ($F = 10.38$, $p = 0.003$), LST ($F = 4.95$, $p = 0.036$), SMST ($F = 4.33$, $p = 0.048$), and whole striatum ($F = 9.02$, $p = 0.006$). Percentage of displacement (change in BP_{ND} between SMCT and MIST PET scans) was not significantly different across groups in any brain region, except in the GP (-5.03 ± 14.6 in CU, compared with 6.15 ± 12.1 in HV; $F = 4.39$, $p = 0.049$). Duration of cannabis use was significantly associated with stress-induced [^{11}C]-(+)-PHNO displacement by endogenous DA in the LST ($r = 0.566$, $p = 0.044$), with no effect in any other brain region. In conclusion, despite an increase in striatal BP_{ND} observed during the control task, chronic cannabis use is not associated with alterations in stress-induced DA release.
Neuropsychopharmacology (2013) **38,** 673–682; doi:10.1038/npp.2012.232; published online 5 December 2012

Keywords: psychosocial stress; dopamine; cannabis use disorder; positron emission tomography; [^{11}C]-(+)-PHNO

INTRODUCTION

Cannabis is the most widely used illicit substance around the world (Bauman and Phongsavan, 1999; Kleber and Dupont, 2012), and the one most commonly used by people with psychosis (Menezes *et al*, 1996; van Os *et al*, 2011). Although many individuals report recreational use of cannabis, a third of individuals experimenting with this drug progress to develop abuse (Gruber and Pope, 2002), and of those who try to quit, an estimated 71% fail within 6 months (Moore and Budney, 2003). Despite the widespread use (World Drug Report 2012), cannabis produces dependence less readily than most other illicit drugs. For example, while 15% and 24% of those who try cocaine and heroin, respectively, develop dependence, only 9% of those who try cannabis develop dependence. Low striatal dopamine (DA) receptor ($D_{2/3}$) availability and low amphetamine-induced DA release in the ventral striatum have been observed with several substance-use disorders, including alcoholism (Martinez *et al*, 2005; Volkow *et al*, 1996), heroin (Martinez *et al*, 2012), cocaine (Volkow *et al*, 1993), and methamphetamine (Volkow *et al*, 2001) use. Less is known about the changes in DA transmission in chronic cannabis use.

The active component of cannabis, Δ^9-THC, is known to cause DA release in both the nucleus accumbens and medial prefrontal cortex in animals (Chen *et al*, 1990; Tanda *et al*, 1997), and there is robust evidence that cannabis can exacerbate pre-existing psychotic symptoms or trigger their re-emergence in those with psychosis (D'Souza *et al*, 2004; Mathers and Ghodse, 1992). Interestingly, an early neuroimaging observation that cannabis increased DA release in a drug-free patient with schizophrenia (Voruganti *et al*, 2001) has been followed by more recent controlled studies showing that Δ^9-THC is not associated with DA release in humans (Stokes *et al*, 2008), despite causing the expected behavioral changes. Consistent with these publications, two recent studies reported no baseline difference in D_2 availability between cannabis users (CU) and healthy volunteers (HV) (Sevy *et al*, 2008; Urban *et al*, 2012), with no effect of cannabis use on amphetamine-induced DA release (Urban *et al*, 2012).

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Received 18 July 2012; revised 23 October 2012; accepted 24 October 2012; accepted article preview online 8 November 2012

Stress is a potent trigger of relapse in addiction (Sinha, 2011; Sinha *et al*, 2011) and an important risk factor for chronic use of cannabis (see Sinha (2011) for review). Furthermore, cross-sensitization between Δ^9-THC and stress have been recently reported in animals (Suplita *et al*, 2008), suggesting that the putative physiological and psychological effects of cannabis could be potentiated in individuals experiencing adverse environmental stress. Epidemiologically, cannabis use at a young age (Andreasson *et al*, 1987; Moore *et al*, 2007) and early experience of adverse (stressful) life events have been associated with increased risk of developing schizophrenia (Read *et al*, 2005), implicating cannabis use and sensitivity to stress as risk factors for psychiatric disorders, particularly schizophrenia. Despite the wealth of research suggesting a strong relationship between stress response and substance use disorders (Robinson and Berridge, 2000), no studies have directly investigated the relationship between the neurochemical response to stress and chronic cannabis use.

The unique binding profile of the DA agonist radiotracer [^{11}C]-(+)-PHNO used in this study includes preferential binding to the D_3 DA receptor subtype, which increases its sensitivity and allows quantification of changes that a $D_{2/3}$ antagonist radiotracer such as [^{11}C]raclopride may not detect. The DA D_3 receptor subtype has lately been the subject of intense interest, due to its postulated involvement in the biochemical mechanism of drug dependence and relapse. Its preferential localization in the mesolimbic DA system in rats and humans, as well as animal studies showing sensitivity of cocaine self-administration to D_3 antagonists and partial agonists, suggest that the D_3 subtype is a key factor in the regulation of motivation, reward, and emotion (Murray *et al*, 1994; for review see Ikemoto and Panksepp, 1999) and thus likely involved in the neurochemical processes underlying substance abuse and dependence.

Based on the potential cross-sensitization between stress and cannabis, and our ability to quantify D_2 and D_3 receptor availability *in-vivo*, here we propose to test the hypothesis that CU during early abstinence have altered dopaminergic responses (increased [^{11}C]-(+)-PHNO displacement) to a validated psychosocial stress challenge (Pruessner *et al*, 2004).

MATERIALS AND METHODS

Subjects

This study was approved by the local Research Ethics Board at the Centre for Addiction and Mental Health (CAMH) and University of Toronto. Thirteen CU with no current or past psychotic disorder and 12 matched HV (assessed by a psychiatrist using the SCID) were recruited from the community through online postings. All subjects signed written informed consent after the study procedures were fully explained.

CU

Inclusion criteria. (1) Male or female between 18 and 40 years old; (2) capacity to provide informed consent in English; (3) no family history (in first-degree relatives) of schizophrenia, schizoaffective disorder, schizotypal personality disorder, or any other disorder involving psychotic symptoms; and (4) regular cannabis use at least three times weekly or meeting DSM-IV criteria for cannabis dependence and positive drug screen both at screening and the days of the positron emission tomography (PET) scans.

Exclusion criteria. (1) Current or lifetime Axis I disorder; 2) current or lifetime treatment with psychotropic medication; (3) substance abuse, other than cannabis, in the past 6 months; and (4) metal implants that would preclude magnetic resonance imaging (MRI).

HV

For HV, inclusion criteria were items (1), (2), and (3), and exclusion criteria were (1)–(4) with item (3) not allowing cannabis use more than five times in the lifetime. Additionally, HV must not have had any personal or family history (first degree relative) of any axis 1 disorder.

Psychopathology Measures

All subjects completed the SCID questionnaire as administered by a trained psychiatrist (RM). In addition, they completed (1) the 12-item Marijuana Craving Questionnaire (MCQ) consisting of four subscales: Compulsivity (evaluating the inability to control cannabis use), Emotionality (evaluating the anticipation of relief from negative mood or withdrawal), Expectancy (evaluation of anticipation of positive outcomes) and Purposefulness (intention and planning to use cannabis for positive outcomes) (Heishman and Singleton, 2006); 2) the 24-item Parental Bonding Instrument (Parker *et al*, 1979) scale, which provides information on parental bonding for subject's mother and father, given its effects on stress-induced DA changes in HV (Pruessner *et al*, 2004); 3) the State-Trait Anxiety Inventory, state version (SAQ; (Spielberger *et al*, 1983) and visual analog scales administered immediately before and after each PET scan.

Montreal Imaging Stress Task

All subjects underwent two PET scans at the same time of the day on two different days, at least 5 days apart: first while undergoing a sensorimotor control task (SMCT) and second while undergoing the Montreal imaging stress task (MIST). To reduce the novelty of the task for the first scan, all subjects performed the non-stressful (SMCT) version of the task before the PET imaging sessions. SMCT scan was always performed first, in order to avoid any residual effects of the stress task. Psychosocial stress was induced using the MIST, which has been validated in previous fMRI and PET studies (Lederbogen *et al*, 2011; Mizrahi *et al*, 2012; Pruessner *et al*, 2004). Briefly, subjects performed mental arithmetic on a computer screen that also displays information about the total number of errors, expected average number of errors, time spent on the current

problem, and performance feedback for each problem (correct, incorrect, timeout). During the stress condition, subjects completed six 6-min block segments of arithmetic while lying in the scanner. The time constraint is adjusted to be slightly beyond each individual's abilities. Because of the manipulation of the difficulty level, the average performance was set at 20–30% correct answers. In addition, subjects were given negative verbal feedback by the investigator for ~2 min between each block, telling them that they need to improve their performance to reach minimum performance requirements. Before the stress task, subjects performed the sensory motor control PET session (non-stress), a similar arithmetic task but without time constraints or negative verbal feedback. In all the experiments, the control or stress task was started ~6–8 min before tracer injection, with 6 min of mathematical questions and ~1–2 min for either neutral or negative feedback and salivary cortisol measurement.

Physiological Measures

Saliva samples were collected every 12 min throughout the experiment. Saliva-derived cortisol was analyzed using a time-resolved fluorescence immunoassay (Dressendorfer *et al*, 1992) and the area under the curve (g/dl/min) was calculated for each subject and each scanning session as described in Pruessner *et al* (2003).

Image and Data Analyses

MRI acquisition. Subjects undertook a standard fast spin echo T1 (FSPGR, TE = 5.3–15, TR = 8.9–12, FOV = 20 cm, matrix = 256 × 256, slice thickness = 1.5, NEX = 1) and a proton density (TE = 17, TR = 6000, FOV = 22 cm, matrix = 256 × 256, slice thickness = 2 mm, NEX = 2) brain MRI acquired on a 1.5T Signa-GE scanner. These images were used for the analysis of the PET scans and to rule out structural lesions.

PET acquisition. Radiosynthesis of [^{11}C]-(+)-PHNO was performed as previously described (Wilson *et al*, 2005). Each subject was administered ~9–10 mCi of tracer (Table 1) and scanned for 90 min. Data were acquired using a high-resolution PET CT scanner, Siemens-Biograph HiRez XVI (Siemens Molecular Imaging, Knoxville, TN, USA) which measures radioactivity in 81 brain sections with a thickness of 2.0 mm each. A custom-fitted thermoplastic mask was made for each subject and used with a head fixation system during PET acquisition to minimize head movement. The images were reconstructed with a 2D filtered back projection algorithm with a ramp filter at Nyquist cutoff frequency.

PET data analysis. Regions of interest (ROIs) were delineated using an automated method implemented in an in-house software (ROMI), abolishing subjectivity in manual ROI drawing (Rusjan *et al*, 2006). We delineated the globus pallidus (GP) and substantia nigra (SN) as per Tziortzi *et al* (2011). Time activity curves from the ROIs were obtained from the dynamic [^{11}C]-(+)-PHNO PET images. PET data were evaluated in the striatal subdivisions based on their functional connections to the limbic, frontal executive, and motor brain regions: limbic striatum (LST, including the ventral striatum), associative striatum (AST, including the pre-dorsal putamen, pre-dorsal caudate, and the post-caudate striatum), and sensorimotor striatum (SMST, post-dorsal putamen) (Martinez *et al*, 2003). Activity from the right and left regions were averaged together, and a weighted average (weighted by subregion volume) was used to derive binding potential (BP_{ND}) with respect to the non-displaceable compartment in the brain for [^{11}C]-(+)-PHNO (cerebellar cortex) applying the simplified reference tissue model (SRTM; Ginovart *et al*, 2007). SRTM provides an estimate of the BP_{ND} of the radiotracer, which is proportional to the more fundamental parameters of receptor number (Bmax) and affinity (1/Kd). Finally, [^{11}C]-(+)-PHNO displacement was calculated as $\%\ \text{Displacement} = \frac{BP_{ND}\ \text{SMCT} - BP_{ND}\ \text{MIST}}{BP_{ND}\ \text{SMCT}} \times 100\%$.

Voxel-wise images were generated using a data-driven method with reference region implemented in DEPICT (Gunn *et al*, 2002). Each parametric map was spatially normalized to an anatomical template (Montreal Neurological Institute) using Statistical Parametric Mapping (SPM) normalization and co-registration tools. Once in the same space, BP_{ND} maps were used to assess significant contrast between conditions (SMCT *vs* MIST) in each group (HV, CU) at the voxel level using an implicit mask of $BP_{ND} > 0.3$. Difference between HV and CU BP_{ND} was evaluated by an independent *t*-test with FWE correction, as implemented in SPM5 (www.fil.ion.ucl.ac.uk/spm).

Statistical Analysis

Independent *t*-test and Chi-square tests were used to detect difference in demographics and injection parameters between groups. Once the SRTM BP_{ND} was obtained for each striatal region, changes between the SMCT and MIST BP_{ND} values were evaluated using paired *t*-tests. Main hypotheses were tested using analysis of variance to investigate differences between HV and CU in SMCT BP_{ND} and stress-induced DA release indexed as [^{11}C]-(+)-PHNO % displacement between groups for each ROI. Subjective perceived stress, PBI measures, MCQ, length of cannabis use, and age of onset, as well as stress-induced cortisol release (quantified as the difference in area under the curve between SMCT and MIST scan) were related to PET data with linear regression analyses. All analyses were two tailed with the conventional $\alpha = 0.05$. ANCOVA with SMCT BP_{ND} as covariate was used to compare percentage of displacement between the CU and HV and obtain estimated marginal means for each group.

RESULTS

Both groups were comparable for demographics (Table 1) and scan parameters (Table 2). All CU and none of the HV met criteria for cannabis dependence and tested positive for cannabis both at screening and on the day of the scans. SMCT and MIST scans were performed on average

Table 1 Demographics of the Study Groups

Demographics (SD)		Healthy volunteers ($n=12$)	Cannabis users ($n=13$)
Age (years)		26.08 (3.8)	24.23 (4.9)
Gender	Male	7	6
	Female	5	7
Mother PBI		33.75 (6.9)	42.62 (7.8)
Smoking status	Non-smoker	11	10
	Smoker	1	3
Cannabis use	Lifetime use (joints)	n/a	7859.85 (10566.6)
	Years of cannabis use	n/a	9.23 (4.9)
	Age at first use (years)	n/a	14.92 (1.3)
Number of joints per week	SMCT	n/a	15.50 (15.5)
	MIST	n/a	14.55 (13.1)
Hours since last joint smoked	SMCT	n/a	9.18 (5.6; range 2–18.5)
	MIST	n/a	11.5 (7.5; range 1–21.5)
Cannabinoids value (μg/l)	SMCT	n/a	1558.7 (2934.2)
	MIST	n/a	2130.2 (3550.3)

Presented as means and standard deviation (SD) showing no significant differences in age ($t=1.042$, df = 1,23, $p=0.308$), gender (chi-square 0.371, $p=0.543$) and smoking status (chi-square 1.009, $p=0.315$). Cannabis users had a significantly higher mother pbi values ($t=-2.998$, df = 1,23, $p=0.006$). In cannabis users, there was no significant difference in any cannabis use parameters between the control (smct) and stress (mist) tasks.

Table 2 PET Scan Parameters Showing No Significant Difference Between Groups or Scan Sessions

Parameter	Healthy volunteers ($n=12$)		Cannabis users ($n=13$)	
	Control task	Stress task	Control task	Stress task
Activity injected (mCi)	9.23 ± 1.65	9.73 ± 0.94	9.98 ± 0.82	10.12 ± 0.43
Specific activity (Ci/mmol)	1019.8 ± 454	1135.7 ± 445	1293.2 ± 500	1338.3 ± 444
Mass injected (μg)	2.50 ± 0.69	2.32 ± 0.62	2.09 ± 0.57	2.06 ± 0.67

12.8 ± 10.1 (HV) and 14.4 ± 7.8 days apart (CU). Out of 13 CU, 7 had no exposure to other drugs, while the remaining 6 reported past occasional use, with no dependence, of MDMA ($n=4$), cocaine ($n=1$), hallucinogenic mushrooms ($n=3$), LSD ($n=1$), heroin ($n=1$), and ketamine ($n=1$). As expected, all subjects performed significantly worse on the MIST (number of errors 42.63 ± 12.2 and 34.92 ± 12.9 for HV and CU, respectively) than in the SMCT (errors 5.23 ± 3.4 and 7.83 ± 5.6 for HV and CU, respectively; $F=104.67$, $p<0.001$ and $F=48.25$, $p<0.001$ for HV and CU, respectively), showing that the MIST was able to adapt to the level of performance of each person and produce a tailored programmed failure within each group. We found no significant difference in the number of errors committed by CU or HV during either the MIST ($t=1.531$, $p=0.139$) or SMCT ($t=-1.388$, $p=0.178$). Following the MIST scan, comparison of post-scan SAQ outcomes revealed that all subjects were less calm ($F=29.99$, df = 4,42, $p<0.001$) and less satisfied ($F=34.35$, df = 1,42, $p<0.001$) but more tense ($F=21.14$, df = 1,42, $p<0.001$), more strained ($F=24.99$, df = 1,42, $p<0.001$), upset ($F=47.38$, df = 1,42, $p<0.001$), and confused ($F=23.62$, df = 1,42, $p<0.001$) compared with the SMCT scan, suggesting that the stress paradigm was effective in eliciting an emotional response. These differences between SMCT and MIST SAQ held true for both the HV and CU subjects independently. Total SAQ scores assessed immediately following the scans were significantly elevated following the MIST as compared with the SMCT ($F=72.80$, df = 1,42, $p<0.001$) and displayed a trend level difference between HV and CU ($F=4.07$, df = 1,20, $p=0.057$).

SMCT BP_{ND} was significantly different between groups in the AST ($F=10.38$, $p=0.004$), LST ($F=4.95$, df = 1,23, $p=0.036$), SMST ($F=4.33$, df = 1,23, $p=0.049$), and whole striatum ($F=9.02$, df = 1,23, $p=0.006$), with HV having lower BP_{ND} as compared with CU in these regions but no difference in D_3-rich regions (GP ($F=2.12$, df = 1,23, $p=0.159$) and SN ($F=0.89$, df = 1,23, $p=0.354$)) (Figure 1, Table 3). Similarly, voxelwise data comparing SMCT BP_{ND} parametric maps between HV and CU showed two clusters of significantly higher BP_{ND} in CU compared with HV at the level of the right caudate/GP and the left putamen (Figure 2). Percentage of displacement was not significantly different across groups in any brain region (Figure 1), except in the GP ($F=4.39$, df = 1.23, $p=0.049$), with CU having less stress-induced changes (CU −5.03%) relative to controls (HV 6.15%). Voxelwise data confirmed the lack of difference between conditions. Interestingly, displacement in the LST was significantly different between CU (3.99%) and HV (−9.11%), when LST SMCT BP_{ND} was taken as a covariate. No other striatal region showed this effect. Years of cannabis use showed significant correlation with stress-induced [^{11}C]-(+)-PHNO displacement in the LST ($r=0.566$, $p=0.04$; Figure 3) and a trend level correlation with [^{11}C]-(+)-PHNO displacement in the entire striatum ($r=0.522$, $p=0.067$); however, these correlations are lost when age was added as a covariate. When SMCT BP_{ND} of the respective regions are taken as a covariate, the correlation with years of use in the LST remained significant ($r=0.789$, $p=0.002$). No correlation was observed between the PET-derived SMCT BP_{ND}, MIST

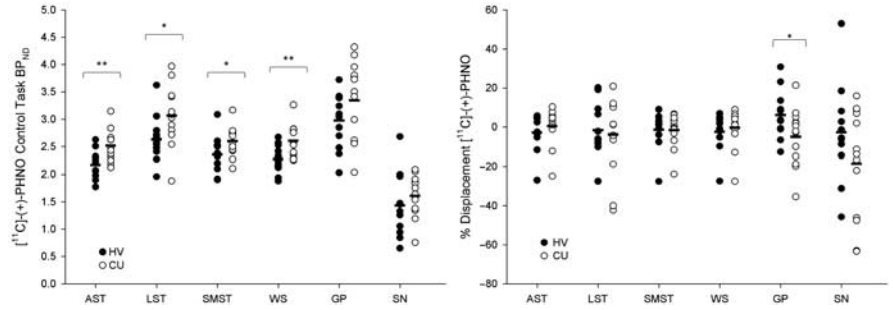

Figure 1 Left: Control task (SMCT) BP_{ND} in HV (filled circles) and CU (empty circles). Right: [^{11}C]-(+)-PHNO displacement in response to the MIST between HV and CU. *$p<0.05$.

Table 3 Binding Potential (BP_{ND}) of [^{11}C]-(+)-PHNO Obtained During the Control (SMCT) and Stress (MIST) Task, and Tracer Displacement in Striatal Subregions, Whole Striatum, Globus Pallidus and Substantia Nigra of HV, CU and CU Who Had No Cannabis Use in the 8 h Preceding the PET Scans

	ROI	SMCT BP_{ND}	MIST BP_{ND}	% Displacement	*t*	*p*
HV	AST	2.17 ± 0.25	2.23 ± 0.31	− 2.87 ± 9.2	− 1.156	0.272
	LST	2.63 ± 0.41	2.64 ± 0.23	− 1.69 ± 13.4	− 0.052	0.959
	SMST	2.35 ± 0.32	2.43 ± 0.39	− 1.35 ± 9.5	− 1.063	0.311
	Striatum	2.28 ± 0.24	2.33 ± 0.30	− 2.41 ± 9.1	− 0.935	0.370
	GP	2.98 ± 0.51	2.80 ± 0.62	6.15 ± 12.1	1.932	0.080
	SN	1.43 ± 0.57	1.46 ± 0.67	− 2.73 ± 24.4	− 0.343	0.738
CU	AST	2.52 ± 0.28	2.50 ± 0.32	0.48 ± 9.4	0.266	0.795
	LST	3.06 ± 0.54	3.11 ± 0.44	− 3.85 ± 19.5	− 0.346	0.735
	SMST	2.60 ± 0.27	2.64 ± 0.31	− 1.69 ± 8.5	− 0.674	0.513
	Striatum	2.60 ± 0.30	2.61 ± 0.34	− 0.40 ± 9.9	− 0.066	0.948
	GP	3.34 ± 0.72	3.45 ± 0.60	− 5.03 ± 14.6	− 0.864	0.404
	SN	1.61 ± 0.38	1.87 ± 0.58	− 18.51 ± 28.1	− 2.188	0.049*
CU >8 h	AST	2.41 ± 0.24	2.48 ± 0.34	− 2.66 ± 10.9	− 0.695	0.510
	LST	2.92 ± 0.54	3.12 ± 0.37	− 10.04 ± 22.5	− 0.935	0.381
	SMST	2.49 ± 0.22	2.60 ± 0.34	− 4.56 ± 9.5	− 1.359	0.216
	Striatum	2.49 ± 0.23	2.58 ± 0.35	− 3.80 ± 11.3	− 0.924	0.386
	GP	3.13 ± 0.76	3.29 ± 0.64	− 7.58 ± 18.0	− 0.819	0.440
	SN	1.48 ± 0.41	1.81 ± 0.60	− 26.27 ± 28.3	− 2.334	0.052

*$p<0.05$, comparison between the MIST and SMCT BP_{ND} value, paired-samples *t*-test.

BP_{ND} or percentage of displacement and PBI scores (maternal, paternal, or total subsets) or total lifetime cannabis use.

PET scans took place on average 9.18 ± 5.6 and 11.5 ± 7.5 h (SMCT and MIST, respectively) since last cannabis use, with 5 of 13 CU subjects reported using cannabis <8 h before the SMCT or MIST scan (Table 1). The PET imaging outcomes from the sample excluding these subjects were analyzed separately to exclude any acute potential effects of cannabis on [^{11}C]-(+)-PHNO binding (Table 3). No differences in BP_{ND} (SMCT or MIST) or tracer displacement were found between HV and this CU group. Additionally, no correlation was observed between hours since last cannabis use and any of the PET outcomes (BP_{ND} or displacement).

We also explored the effects of cannabis use as assessed with the MCQ on both BP_{ND} and stress-induced BP_{ND} change. We only found a significant correlation between change in compulsivity to use cannabis following the control scan and GP BP_{ND} ($r=0.644$, $p=0.018$) during SMCT. On the day of the stress scan, greater pre- and post-scan MCQ emotionality were significantly associated with high LST BP_{ND} values ($r=0.581$, $p=0.037$ and $r=0.563$, $p=0.045$, respectively). Lower stress-induced [^{11}C]-(+)-PHNO displacement in the GP ($r=-0.656$, $p=0.015$) and trend level in the SN ($r=-0.513$, $p=0.073$) were significantly associated with greater MCQ compulsivity, as assessed before the MIST scan. A greater magnitude of cortisol response to stress was related to the greater displacement of [^{11}C]-(+)-PHNO in the AST ($r=0.426$, $p=0.034$), but not in any other regions, when all subjects are considered. Stress-induced cortisol release was not significantly different between groups (HV −14.76 ± 19.3, CU 2.80 ± 23.4; $F=4.15$, $p=0.053$). Additionally, in CU,

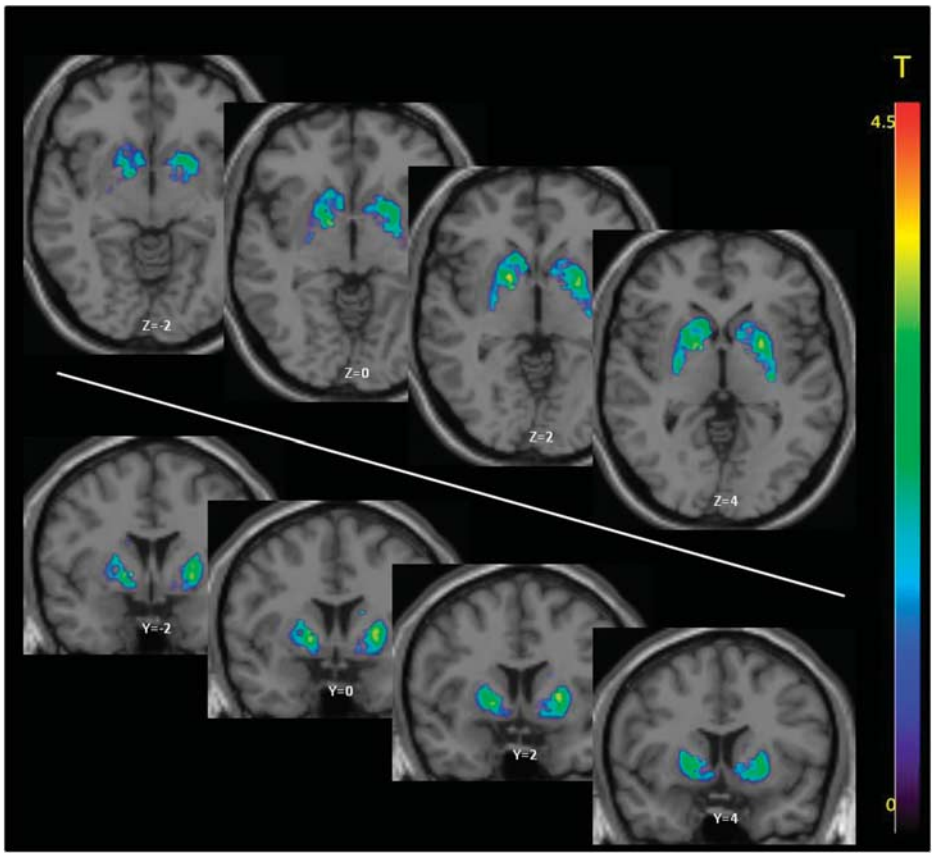

Figure 2 Voxelwise comparison between SMCT difference between HV and CU. Images were generated using DEPICT, with voxels showing significantly higher BP_{ND} in CU compared with HV (FWE-corrected, $p<0.05$) overlaid on standard MRI template. Two clusters of significant voxels were detected: a cluster encompassing 455 voxels in the right hemisphere with significant voxels in the right globus pallidus (MNI coordinates [16, 0, −2]; corrected $p=0.032$, uncorrected $p=0.005$) and right caudate (455 voxels, MNI coordinates [14, 10, 14]; corrected $p=0.032$, uncorrected $p=0.005$); and a 489 voxel cluster in the left putamen (MNI coordinates [−26, −12, 8]; corrected $p=0.024$ uncorrected $p=0.003$).

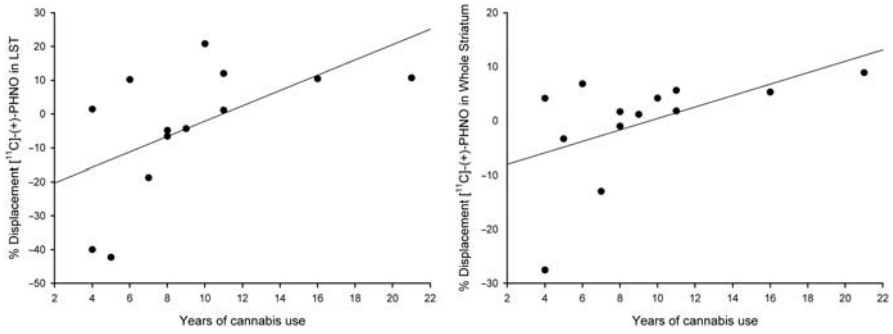

Figure 3 Relationship between years of use and percentage of displacement of [^{11}C]-(+)-PHNO, showing significant correlation in LST (left panel; $r=0.566$, $p=0.04$) and a trend level relationship in the entire striatum (right panel; $r=0.522$, $p=0.067$). The correlation with years of use in the LST remains significant ($r=0.789$, $p=0.002$) when the percentage of displacement is corrected for the LST SMCT BPND.

increased stress-induced cortisol release correlated with higher change in MCQ expectancy and purposefulness ($r=0.809$, $p=0.001$ and $r=0.736$, $p=0.004$, respectively).

DISCUSSION

Using [^{11}C]-(+)-PHNO PET imaging and a validated psychosocial stress paradigm to measure tracer displacement, we observed no difference in stress-induced DA release between HV and chronic CU. Our findings support previous observations by Urban *et al* (2012), who found no difference between CU and HV in [^{11}C]-raclopride binding following an acute amphetamine challenge. We did observe increased BP_{ND} in D_2-rich areas in CU compared with HV during the performance of the control (SMCT) task. Taken together, these findings suggest that chronic cannabis use does not show a different DA response to stress.

DA agonist radiotracer [^{11}C]-(+)-PHNO demonstrates an estimated ~20-fold higher affinity for D_3 *vs* D_2 *in vivo*

(Narendran *et al*, 2006b; Rabiner *et al*, 2009). Detected regional signal following [^{11}C]-(+)-PHNO administration is therefore a function of the differential affinity as well as concentration of D_3 *vs* D_2 receptors in a given region. In D_3-rich regions like the GP, D_3 binding is thought to account for ~67% of the [^{11}C]-(+)-PHNO signal, while the SN represents 100% D_3 binding; thus, its signal can be a sole marker of D_3 effects (Searle *et al*, 2010; Tziortzi *et al*, 2011). In other regions like the dorsal striatum (caudate, AST and putamen, SMST), the relative concentration of D_2 receptors is much higher and, therefore, only a small component of the signal, 10–40%, is attributable to D_3 (Searle *et al*, 2010; Tziortzi *et al*, 2011). Consequently, observed elevated BP_{ND} suggest that chronic cannabis use may result in increased receptor availability in D_2-rich regions (AST, LST, SMST and the whole striatum), while the D_3-rich regions are relatively unaffected. However, any conclusions derived from the comparison of SMCT BP_{ND} measurements between groups are confounded by the fact that the SMCT scan was obtained while the subjects were performing a cognitive task, which cannot be considered a 'true' baseline state. In contrast to our findings, increased [^{11}C]-(+)-PHNO BP_{ND} was reported in D_3-rich areas of chronic methamphetamine users (Boileau *et al*, 2012), which suggests that chronic use of dopaminergic drugs affects the brain in a distinct fashion from cannabis. Nevertheless, it is worth considering that although the presence of D_3 autoreceptors in all SN DA neurons is well established (Diaz *et al*, 2000), there is still no clear evidence of a physiological role of these receptors in SN (Davila *et al,* 2003). The higher [^{11}C]-(+)-PHNO BP_{ND} in CU during the control SMCT task in D_2-rich regions could be interpreted as an increase in $D_{2/3}$ receptor availability, reflecting either lower levels of endogenous DA (while performing a cognitive task, SMCT) or upregulation of D_2 receptors. Recent studies evaluating the effect of chronic exposure to THC in animals using ^{3}H-labeled version of (+)-PHNO have observed increased tracer binding, accompanied by increased D_2 and D_3 densities in the midbrain (Ginovart *et al*, 2012). Previous reports using [^{11}C]-raclopride imaging in humans have demonstrated no change in (true) baseline striatal $D_{2/3}$ receptor availability in chronic CU (Sevy *et al*, 2008; Stokes *et al*, 2012; Urban *et al*, 2012).

We found that the MIST was able to elicit a significant emotional stress response as indicated by the significantly elevated total SAQ scores following the MIST as compared with the SMCT. However, despite the increase in subjective report of stress following the MIST, we did not find a significant change of [^{11}C]-(+)-PHNO BP_{ND} between SMCT and MIST scans in the HV (Table 3). Our finding is consistent with previous observations using the same psychosocial stress paradigm (Pruessner *et al,* 2004), in which the MIST elicited a significant change in $D_{2/3}$ receptor binding but only in certain type of individuals, such as those with a history of low maternal care or with certain personality types (Suridjan *et al*, 2012).

The lack of effect on stress-induced DA release distinguishes chronic cannabis use disorder from alcoholism, stimulant, and heroin dependence, which have all been shown to reduce amphetamine-induced DA release (Martinez *et al*, 2007; Martinez *et al*, 2012; Volkow *et al*, 1993; Volkow *et al*, 1996). However, our findings of lack of difference in tracer displacement in response to stress are remarkably consistent with a recent study in chronic CU (Urban *et al*, 2012). Previous studies combining [^{11}C]raclopride PET and *in vivo* microdialysis in rhesus monkeys have shown that very large increases in synaptic DA concentrations were reflected in comparatively smaller changes in [^{11}C]raclopride binding (Breier *et al*, 1997; Endres *et al*, 1997). [^{11}C]-(+)-PHNO is more sensitive to displacement by synaptic DA (Ginovart *et al*, 2006; Narendran *et al*, 2006a; Shotbolt *et al*, 2012). However, the relationship between synaptic DA outflow and [^{11}C]-(+)-PHNO binding is unknown. It is therefore still possible that there was a significant difference in stress-induced DA release between the CU and healthy controls that did not translate into a significant difference in [^{11}C]-(+)-PHNO percentage of displacement.

We observed no correlation between the age of onset of cannabis use and displacement of [^{11}C]-(+)-PHNO in any of the regions studied. We did, however, find that the length of cannabis use (in years) correlated with stress-induced tracer displacement in the LST and whole striatum. This observation topographically corresponds to the dopaminergic alterations commonly reported in substance abuse, but the relationship, if replicated in a larger cohort, would suggest that chronic cannabis use sensitizes, rather than blunts, striatal dopaminergic signaling. Correlation between DA receptor availability (indexed as SMCT BP_{ND}) in LST with MCQ emotionality (cannabis use to alleviate negative mood) provides additional support for the involvement of ventral striatum in regulating aspects of substance use. Interestingly, significant elevations in the emotionality component of the MCQ has also been observed following the Trier Social Stress Task (McRae-Clark *et al*, 2011), while recent experience monitoring studies have linked social anxiety with cannabis cravings outside the laboratory environment (Buckner *et al*, 2012). The correlation between cortisol release and expectancy and purposefulness measures of the MCQ validates the linkage between stress and cannabis craving. Although a recent large prospective population study reported reduced cortisol stress response to a stress task in CU (van Leeuwen *et al*, 2011), we observed no difference in cortisol release between groups, likely due to the sample size.

Some limitations are typical in neurochemical brain imaging studies in humans. First, while abstinence from cannabis was not an inclusion criteria, out of 13 scanned subjects, 5 subjects have used cannabis in the 8 h before the SMCT or MIST scan. Although this acute use could conceivably affect the stress-response reported here, previous studies have shown, at best, a modest effect of acute cannabinoid administration on DA release (Bossong *et al*, 2009; Stokes *et al*, 2012). When those five subjects are excluded from the analysis, the main finding, that is the lack of a difference between CU and HV on the stress-induced DA release, remains unchanged (except for the loss of significance in the GP displacement; Table 3). Thus, either 2–3 weeks withdrawal (Urban *et al*, 2012) or current chronic use seem to produce similar effects on the DA system. The inclusion of an additional group composed of former CU with 2–3 months of abstinence would possibly rule out intoxication or short-term withdrawal effects and thus help differentiate between the 'trait' and 'state' aspects of

cannabis use and their effect on the DA system. Second, as commonly observed with CU, 6 out of 13 CU reported past use of other drugs. Excluding these subjects from the study did not affect the main finding (except again for the GP displacement which is lost, suggesting a possible weak effect). Third, given that our HV group did not show a significant DA response to stress, an assertion of a 'blunted' or 'sensitized' DA response to stress cannot be entertained. Fourth, baseline estimates of D_2 and D_3 binding were not 'true' baseline measures, as subjects were performing the cognitive (non-stressful) version of the MIST. Fifth, it has recently been suggested that [^{11}C]-(+)-PHNO may not be at tracer dose in the D_3-rich regions, which would hinder its accurate quantification (Gallezot *et al*, 2012; Rabiner and Laruelle, 2010). However, our significant findings were relatively specific to D_2 regions, and scan sessions (SMCT and MIST) were carried out on separate days, at least 5 days apart (except for one subject who could not come on two occasions). Additionally, the nature of the stress task did not allow for the order of the scans to be randomized. With all the subjects in the study, SMCT control task was done during the first PET scan, followed by the stress task (MIST) during the second scan. The use of the social stress paradigm to induce DA release is appropriate for use in populations who have not been exposed to dopaminergic drugs and is therefore a safe alternative compared with studies performing imaging following amphetamine or methylphenidate administration. Finally, the lack of effects in the ventral striatum of the acute stress challenge may be related to the joint [^{11}C]-(+)-PHNO binding to D_2 and D_3 receptors, which may increase sampling variability in this brain region (Narendran *et al*, 2006b).

In conclusion, the present work supports previously published observations that chronic cannabis use, unlike other addictions, does not affect DA release. Differences in BP_{ND} between the CU and HV during the control task provide support for future *in vivo* studies quantifying changes in striatal D_2 receptor expression in chronic cannabis use. Additionally, our findings in the SN and GP suggest the need for further examination using larger samples of changes in D_3 receptor expression and availability in cannabis use and in other substance-use disorders.

ACKNOWLEDGEMENTS

This work is supported by an operating grant from the Ontario Mental Health Foundation (OMHF). RM is supported by the New Investigator Award from Canadian Institute of Health Research (CIHR) and the OMHF New Investigator Fellowship. The initial pilot data was also supported by the Connaught New Staff matching grant and the Dean's fund from the University of Toronto. TPG was supported by the Chair in Addiction Psychiatry at the University of Toronto. We also wish to thank Armando Garcia, Winston Stableford, Peter Bloomfield and Matthew Mitchell (Siemens Molecular Imaging, Knoxville, TN, USA) for their technical assistance, Dr. Isabelle Boileau for valuable discussion, and Dr. Jens Pruessner for the development of the MIST.

DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)